

## PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY  
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 21369WO	<b>FOR FURTHER ACTION</b> See Form PCT/PEA/416	
International application No. PCT/EP2004/001173	International filing date (day/month/year) 05.02.2004	Priority date (day/month/year) 05.02.2003
International Patent Classification (IPC) or national classification and IPC C12N1/14		
Applicant DSM IP ASSETS B.V. et al.		
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input type="checkbox"/> <i>(sent to the applicant and to the International Bureau)</i> a total of sheets, as follows:</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</li> <li><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</li> </ul> <p>b. <input type="checkbox"/> <i>(sent to the International Bureau only)</i> a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>		
<p>4. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> <li><input checked="" type="checkbox"/> Box No. I Basis of the opinion</li> <li><input type="checkbox"/> Box No. II Priority</li> <li><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</li> <li><input type="checkbox"/> Box No. IV Lack of unity of invention</li> <li><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</li> <li><input type="checkbox"/> Box No. VI Certain documents cited</li> <li><input checked="" type="checkbox"/> Box No. VII Certain defects in the international application</li> <li><input type="checkbox"/> Box No. VIII Certain observations on the international application</li> </ul>		
Date of submission of the demand 13.08.2004	Date of completion of this report 02.02.2005	
Name and mailing address of the international preliminary examining authority:   European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer  Lopez García, F Telephone No. +49 89 2399-2171	



**INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITY**

International application No.  
PCT/EP2004/001173

**Box No. I Basis of the report**

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
  - This report is based on translations from the original language into the following language, which is the language of a translation furnished for the purposes of:
    - international search (under Rules 12.3 and 23.1(b))
    - publication of the international application (under Rule 12.4)
    - international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements\*** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

**Description, Pages**

1-26 as originally filed

**Claims, Numbers**

1-11 as originally filed

**Drawings, Sheets**

1/9-9/9 as originally filed

- a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

- The amendments have resulted in the cancellation of:
  - the description, pages
  - the claims, Nos.
  - the drawings, sheets/figs
  - the sequence listing (*specify*):
  - any table(s) related to sequence listing (*specify*):
- This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
  - the description, pages
  - the claims, Nos.
  - the drawings, sheets/figs
  - the sequence listing (*specify*):
  - any table(s) related to sequence listing (*specify*):

\* If item 4 applies, some or all of these sheets may be marked "superseded."

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**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

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**1. Statement**

Novelty (N)	Yes:	Claims	9,10
	No:	Claims	1-8, 11
Inventive step (IS)	Yes:	Claims	10
	No:	Claims	1-9, 11
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

**2. Citations and explanations (Rule 70.7):**

**see separate sheet**

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**Box No. VII Certain defects in the international application**

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The following defects in the form or contents of the international application have been noted:

**see separate sheet**

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**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

D1:WO 00/50576 A

D2:RUIJTER G J G ET AL: 'OXALIC ACID PRODUCTION BY ASPERGILLUS NIGER: AN OXALATE-NON-PRODUCING MUTANT PRODUCES CITRIC ACID AT PH 5 AND IN THE PRESENCE OF MANGANESE' MICROBIOLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, READING, GB, vol. 145, no. 9, September 1999 (1999-09), pages 2569-2576, XP000905457 ISSN: 1350-0872

D3:HOMBERGH VAN DEN J P T W ET AL: 'NEW PROTEASE MUTANTS IN ASPERGILLUS NIGER RESULTS IN STRONGLY REDUCED IN VITRO DEGRADATION OF TARGET PROTEINS; GENETICAL AND BIOCHEMICAL CHARACTERIZATION OF SEVEN COMPLEMENTATION GROUPS' CURRENT GENETICS, NEW YORK, NY, US, vol. 28, no. 4, 1995, pages 299-308, XP000867320 ISSN: 0172-8083

D4:WO 97/10350 A

D5:PEDERSEN H ET AL: 'Construction and characterization of an oxalic acid nonproducing strain of Aspergillus niger.' METABOLIC ENGINEERING. UNITED STATES JAN 2000, vol. 2, no. 1, January 2000 (2000-01), pages 34-41, XP002286173 ISSN: 1096-7176

D6:US-B1-6 432 672

D7:US-A-4 519 697

D8:SARANGBIN S ET AL: 'Yam bean starch: a novel substrate for citric acid production by the protease-negative mutant strain of Aspergillus niger' CARBOHYDRATE POLYMERS, APPLIED SCIENCE PUBLISHERS, LTD. BARKING, GB, vol. 38, no. 3, March 1999 (1999-03), pages 219-224, XP004154847 ISSN: 0144-8617

The present application is directed to oxalate deficient *A. niger* strains and UV-mutagenesis methods for its selection.

**1. Novelty (Art. 33(2) PCT).**

Oxalate deficient *Aspergillus niger* are known from the prior art (D1-D5, see passages of the ISR). As the subject-matter of claim 1 does not specify the enzyme expressed, it

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is assumed that the microorganisms of D1-D5 produce many enzymes at least at the same amount as the parent strain. The microorganisms of D1-D5 fulfill the requirements of claims 1-4, 6-8 which are hence not novel.

The microorganism of D1 is directed to the production of heterologous proteins (see claims 24-26). Therefore, claim 5 is also not novel.

The use claim 11 is also not novel over D1-D5.

**2. Inventive step (Art. 33(3) PCT).**

**2.1 Inventive step of the microorganisms of claims 1-8.**

2.1.1 D1 and D5 are considered the closest prior art. They relate to the production of homologous/heterologous proteins in oxaloacetate hydrolase deficient *A. niger*. Both documents are aware of the problems that oxalate production generates with downstream processing of industrial enzymes.

The present application has the same aim as D1 and D5 with the requirement that the microorganisms produce at least the same amount of enzyme as the parent strain. However, this requirement seems to be obvious for the skilled person wishing to provide alternative processes for the production of proteins. Therefore, the subject-matter of claims 1-8 is not inventive.

2.1.2 D8 provided protease-negative mutants of *A. niger* having enhanced glucoamylase and citric acid production. Said mutant were selected after UV-mutagenesis.

The combination of D8 with the teaching of D1 or D5 regarding the advantages of providing oxalate-deficient strains, would lead to the skilled person to further select those strains having reduced oxalate production.

Therefore, claims 1-8 are also not inventive over the combination of D8 with D1 or D5.

**2.2 Inventive step of the method of claims 9 and 10.**

D8 is considered the closest prior art. The preparation of an oxalate deficient

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microorganisms from those of D8 would be obvious to the skilled person in the knowledge of D2 or D3, wherein said kind of mutants have been obtained by selection after UV-mutagenesis.

Therefore, the subject-matter of claims 9 is not inventive over D8 in combination with D2 or D3.

However, nowhere it is disclosed or suggested the advantages of having a residual OAH activity. Therefore, claim 10 is considered to be inventive.

**3. Industrial applicability (Art. 33(4) PCT).**

The subject-matter of claims 1-11 is industrially applicable.

**Re Item VII**

**Certain defects in the international application**

1. Claims 1-4 and 6 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved, which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result.

2. The microorganisms of claims 1-8 do not meet the requirements of Art. 5 PCT, since the artificial mutation processes to which the microorganisms were subjected (UV-mutagenesis) are not reproducible (see PCT Guidelines 4.13) and on the other hand, the mutated microorganisms have not been deposited according to Rule 13bis PCT.

3. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2-D4, D7 and D8 is not mentioned in the description, nor are these documents identified therein.